Response of estuarine and freshwater macroinvertebrate assemblages to habitat, water quality, flow and land use change in the lowland Amatikulu/Nyoni catchment

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Submitted in fulfilment of the academic requirements for the degree of

Master of Science

in the Discipline of Ecological Sciences

School of Life Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg Campus

2018

ABSTRACT

Water has become a scarce commodity in Sub-Saharan Africa and in drought prone South Africa. Due to the extensive utilization of water resources in South Africa, management and monitoring of our rivers and estuaries is required by law to achieve a balance between use and protection. Macrobenthic invertebrates of estuaries and freshwater macroinvertebrates have played a large role as indicators of ecosystem health. The lowland Amatikulu/Nyoni catchment in the province of KwaZulu-Natal is relatively poorly understood and baseline information about the ecology of the ecosystem is required. This therefore advocates more research into the Amatikulu/Nyoni catchments ecological wellbeing is needed to inform best management practices. The overall aim of this study was to utilize aquatic macroinvertebrates as ecological indicators to evaluate the current biological condition of the Amatikulu/Nyoni River/Estuary. Freshwater and estuarine invertebrates were evaluated separately in the study with two main lines of evidence including: (1) the use of valid statistical methods to determine how water quality, habitat and sediment composition affected estuarine and freshwater macroinvertebrates community structure, abundance and distribution, and (2) the use of an established community metric measure or biological index namely the South African Scoring System (SASS) Version 5 to evaluate the wellbeing of the freshwater invertebrate communities. The application of these lines of evidence are detailed in chapters two and three.

Chapter one is a general introduction for the thesis and a comprehensive literature review of how and why macroinvertebrates have been used as a bio indicators of freshwater and estuarine integrity. Chapter two describes the collection of freshwater macroinvertebrates from four freshwater sites and their assessment using the SASS5 community metric measure during the high and low flow seasons in 2017. In addition, multivariate statistical analyses were

performed on the data to test the significance of macroinvertebrate community shifts and correlations with changes in temperature, conductivity, dissolved oxygen, percentage dissolved oxygen, pH, South African Scoring System Version 5 Scores, Average Score Per Taxa (ASPT), the number of individuals for each survey and flow periods and between sites. The lowland Amatikulu River catchment sites showed that there was a change in the ecological condition of freshwater macroinvertebrate assemblages as well as along a longitudinal gradient with upstream sites having better conditions to support more sensitive species.

In the third chapter, estuarine benthic macroinvertebrates were collected from four estuary sites using a Van Veen grab during two sampling surveys in 2017. To analyse the data, Conoco version 4.5 was also used to test the significance of benthic invertebrate community shifts and correlations with environmental, spatial and temporal variables. Estuarine benthic invertebrate assemblages varied between sampling sessions in accordance with estuary mouth conditions, nutrient concentrations, sediment grain size distributions and total organic content levels compared with other environmental parameters. By identifying the relationship and drivers between aquatic macroinvertebrates with water quality, habitat, flow and anthropogenic land use change, insight has been gained into the structure and function of the Amatikulu/Nyoni Catchment, its ecosystem health and some management and monitoring recommendations derived to contribute to the sustainable management of the system.

Keywords: Amatikulu, catchment, freshwater, estuary, macroinvertebrates, water quality.

PREFACE

The data described in this thesis were collected in the lowland Amatikulu River catchment, KwaZulu-Natal Province, Republic of South Africa, from April 2017 to September 2017. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Dr Gordon C. O'Brien and Professor Colleen T. Downs.

This thesis, submitted for the degree of Master of Science in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, School of Life Sciences, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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Mbalenhle Thabile Sosibo
January 2018

I certify that the above statement is correct and as the candidate's supervisor I have approved this thesis for submission.

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Dr Gordon C. O'Brien

Supervisor

January 2018

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Professor Colleen T. Downs

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DECLARATION 1 - PLAGIARISM

I, Mbalenhle Thabile Sosibo, declare that

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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

Publication 1- in preparation Chapter 2

Response of Macroinvertebrates to water quality, quantity and habitat condition changes in the lowland Amatikulu River catchment, KwaZulu-Natal, South Africa.

MT Sosibo, CT Downs & GC O'Brien

Author contributions:

MS conceived paper with CTD and GCO. MS collected and analysed data, and wrote the paper. CTD and GCO contributed valuable comments to the manuscript.

Publication 2-- in preparation Chapter 3

Response of macrobenthic invertebrates to water quality, quantity and habitat condition changes in the Amatikulu/Nyoni Estuary, KwaZulu-Natal, South Africa

MT Sosibo, CT Downs & GC O'Brien

Author contributions:

MS conceived paper with CTD and GCO. MS collected and analysed data, and wrote the paper. CTD and GCO contributed valuable comments to the manuscript.

Signed: ……

Mbalenhle Thabile Sosibo
January 2018

ACKNOWLEDGEMENTS

A special thanks to my supervisors Dr Gordon C. O'Brien and Professor Colleen T. Downs both academically and financially, this project would not be possible without them. I would like to acknowledge the National Research Foundation (ZA) and the South African pulp and paper industry (SAPPI) for provision of research funds and making this research possible. This thesis is dedicated to my parents and siblings. Thank you for continuously supporting me emotionally, physically and financially. I would not have been able to get through this without your encouragement.

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CHAPTER 1

INTRODUCTION

Water is utilised by multiple stakeholders such as heavy and light industries, commercial agriculture and domestic use, in many catchments their level of use is unsustainable especially in arid developing regions of the world (Ollis *et al*., 2006). These activities have been linked to the degradation and low water quality of water bodies such as rivers and estuaries (Buck *et al*., 2004). Agricultural activities contribute to increased nutrients and sediment loads in water bodies. The water quality of a water body is also dependent on the scale and magnitude of the activities that are in and around them (Buck *et al*., 2004). If there are other activities such as urban and peri urban communities and waste disposal in close proximity to the water body, they may contribute to a decline in water quality (Buck *et al*., 2004). The cumulative effects of different activities contribute to the degradation in water quality, therefore, is of not at primary importance to regional water resource management (King and Pienaar, 2011).

Vegetation within and surrounding water bodies (riparian vegetation) affects the quantity that enters them and quality of the water within water bodies (Tong and Chen, 2002, Buck *et al*., 2004). The type of vegetation that surrounds the water bodies can influence the surface water temperature; hydrologic cycle and water balance by evapotranspiration and percolation to mention a few (Tong and Chen, 2002). Agricultural land is generally enriched with sediment and nutrients when it rains resulting in surface runoff, the residue from the land is carried into the river/estuary waters (Lenat and Crawford, 1994). Some evidence demonstrates that agricultural activities have been documented to have a high negative effect on water bodies in comparison to other land use activities such as forestry and urban areas (Lenat and Crawford, 1994; Tong and Chen, 2002). The increased sediment load from agricultural activities results in the degradation

of habitats for fish and invertebrates (Tong and Chen, 2002). Agricultural activities affect 90% of the world's freshwater supply and with an increasing demand (Scanlon *et al*., 2007).

Freshwater systems have been well documented to be amongst the most threatened and overutilised ecosystems in the world, their integrity is a representation of the catchment conditions (Iliopoulou-Georgudaki *et al*., 2003; Ollis *et al*., 2006). Rivers are not only essential to human life, they are also essential for supporting many animals (Vaughan and Ormerod, 2014). With an increase in human activity on a landscape scale, there are threats to the ecological status of freshwater systems (Roy *et al*., 2003). Different land use activities have different effects on river health on a spatial and temporal scale (Allan, 2004; Miserendino *et al*., 2011). Activities such as: agriculture; contaminant pollution; hydrologic action; riparian clearing and sedimentation may alter the chemical and biotic conditions of the system (Allan, 2004). Additionally, these activities may alter the food source and habitat quality for biota within the systems and this results in the degradation of the biotic community (Roy *et al*., 2003). Land use activities have also resulted in the degradation of river systems by altering water quality; habitat and macroinvertebrate communities (Roy *et al*., 2003).

Estuaries are very complex and dynamic ecosystems that host a large diversity of biota (Borja *et al*. 2008). They are a transitional zone whereby freshwater from rivers and streams meet the ocean (Cardoso *et al*., 2008). They are also varying in chemical and physical attributes spatially and temporally (Breen and McKenzie, 2001). These ecosystems provide multiple habitats for fauna and flora such as fish at different life stages and benthic invertebrates (Adams, 2014). Estuaries provide services which have high economic value and there are other services that cannot be assigned an economic value due to their immeasurable value (Cardoso *et al*., 2008). Due to increased land use activities driven by economic and social development, estuarine

ecosystems have also been put under stress and this affects the faunal assemblages of species by altering the availability of habitats and resources (Borja *et al*., 2008). Land use activities such as agriculture and heavy/light industry which extract resources from the estuaries or deposit chemical discharge into the system, which then result in sedimentation, nutrient loading, and water contamination (Dauer *et al*., 2000; Borja *et al*., 2008).

Management and monitoring programs with policies are needed to make sure aquatic ecosystems are not used excessively. Monitoring of the ecosystem condition is important for informing management practices, they allow managers to determine which management actions should be taken (King and Pienaar, 2011). Globally bioindicators have been used to inform environmental management practices (Azrina *et al*., 2006; Shafie *et al*., 2017). Using the biotic community to assess river and estuarine integrity is a widespread method in the aquatic research community (Fore *et al*., 1996; Ollis *et al*., 2006). Biological indicators of river/estuary health include macroinvertebrates and fish communities (Ollis *et al*., 2006). Macroinvertebrates are sensitive to changes in the ecosystem, the decrease in variety and abundance of species may represent a decline in habitat quality and availability (Allan, 2004). Aquatic invertebrates compared with other aquatic biota, are considered the most suitable bioindicators (Chutter, 1994; Iliopoulou-Georgudaki *et al*., 2003).

1.1 The use of aquatic invertebrates as ecological indicators/bioindicators

Aquatic macroinvertebrates give template snapshot into what is happening in the ecosystem. Aquatic invertebrates are important in determining the health of an aquatic system especially since they are vulnerable to water pollution and changes, furthermore physiochemical information on a system is insufficient on its own (Chutter, 1994; Iliopoulou-Georgudaki *et al*.,

2003; Poulton *et al*., 2003; Arimoro and Ikomi, 2009; Wahizatul *et al*., 2011). In other terms aquatic invertebrates are used to monitor the functionality and structure of water bodies (Karr, 1999; Iliopoulou-Georgudaki *et al*., 2003). Macroinvertebrates are one of the most popular organism groups used as bioindicators and to monitor river health in freshwater river systems because they react to both natural and anthropogenic induced changes, they are also quite accessible compared with other groups (Chutter, 1994; Iliopoulou-Georgudaki *et al*., 2003; Poulton *et al*., 2003; Azrina *et al*., 2006; Ollis *et al*., 2006; Arimoro and Ikomi, 2009).

Different taxa have different responses to changes in environmental factors such as water quality and habitat availability (Ollis *et al*., 2006). Tolerant or less sensitive taxa may be found in unfavourable (stressed) environmental conditions compared with taxa that are sensitive and require specific conditions to thrive (Ollis *et al*., 2006; Wahizatul et al., 2011). The sensitive species such as Ephemeridae (mayflies) in aquatic invertebrate communities react faster to stress than more tolerant species such as Chironomidae (midges) (Iliopoulou-Georgudaki *et al*., 2003; Ollis *et al*., 2006). When the sensitive species abundance and variability decrease this is an indication of environmental stress or degradation (Ollis *et al*., 2006), this can be used to evaluate the condition of ecosystem components.

1.2 Aquatic invertebrate community metrics and lines of evidence in rivers

To perform assessments on aquatic ecosystems, a multimetric index approach has been used all around the world for aquatic research (Karr, 1999). Initially the most detailed multimetric indices were developed around fish, but over time aquatic invertebrates were also included (Fore *et al*. 1996). The sensitivity of aquatic macroinvertebrate taxa to different ecosystem alterations (e.g. organic enrichment) is the main factor for the formulation of their best multimetric indices (Karr,

1999). Multimetric indices further incorporate multiple factors of a system to give a more realistic picture of what condition the system is in (Karr, 1999). Biotic indices using aquatic invertebrates are well established and many have been developed in different parts of the world (Ollis *et al*., 2006). In the United Kingdom (UK), Woodiwiss (1964) developed a water quality scoring system known as the Trent Biotic Index (TBI) which is based on aquatic macroinvertebrates to determine the ecosystem health. The TBI later was not accurate enough and the Biological monitoring working party scoring system (BMWP) was developed for the UK (Armitage *et al*., 1983). In France the macroinvertebrate biomonitoring based MultiMetric Invertebrate Index (I2M2) has been used and tested (Mondy et al., 2012). There are many more multimetric indices based on aquatic macroinvertebrates used outside of Europe (De Pauw and Vanhooren, 1983; Stark, 1985; Barbour *et al*., 1999; Thirion, 2007).

Bioassessment indices have been successfully and widely used in their respective countries, however they require supplementary information to make them more reliable (Ollis *et al*., 2006). They are generally limited in showing minor changes such as degradation at smaller scales (Ollis *et al*., 2006). The application of multiple indices on one project may produce conflicting results, however the aquatic invertebrates have still proven to be good ecosystem health indicators (Iliopoulou-Georgudaki *et al*., 2003). Index scores are affected by the presence and the absence of taxa (Iliopoulou-Georgudaki *et al*., 2003). Biotic indices give a qualitative feedback, however they do not reflect quantitative changes in the system (Iliopoulou-Georgudaki *et al*., 2003).

In the last few decades South Africa has had its own indices developed for bioassessments. In 1972 the Chutter's Biotic index (CBI) was developed to measure the degree of organic pollution but only in the stones-in-current habitat (Ollis *et al*., 2006). The CBI ranked

sensitive and tolerant taxa found from 0 (very sensitive) to 10 (very tolerant) with abundance and variability to determine the extent of organic pollution in a system (Ollis et al., 2006). Using only one habitat limits the quality of data for a diverse system, the CBI was also not widely used due to it being expensive and time consuming (Ollis *et al*., 2006).

The South African Scoring System (SASS) developed over many years using macroinvertebrate community attributes as indicators of river health, it is a cheap and rapid bioassessment method that has been widely used in South Africa (Chutter, 1994, Dickens and Graham, 2002, Bowd *et al*., 2006). The SASS method has been used to show a trend of water quality change over time. Like the CBI bioassessment method, SASS has preselected taxa used to determine the ecological integrity of a river system (Dickens and Graham, 2002, Ollis *et al*., 2006). In the latest version SASS5, the different taxa have also been allocated sensitivity scores (1 being very tolerant to 15 being very sensitive) based on their response to pollution and disturbance (Ollis *et al*., 2006). Macroinvertebrates are collected using a kick net in three different biotopes. The three biotopes include Vegetation (marginal and aquatic), Stones (in and out of current) and GSM (gravel, sand and mud) which is a good representation of the instream habitat.

To evaluate the ecological integrity of a river, the three main indices obtained from SASS are: average score per taxon (ASPT), SASS5 score, and the number of taxa (Dickens and Graham, 2002, Ollis *et al*., 2006). River sites are further assessed within their respective Ecoregions whereby a biological banding system developed by the Institute of Natural Resources (INR), it is used to interpret bioassessment data (Dallas, 2007). The biological bands range from A (Unmodified or natural) to F (Critically or extremely modified). To determine which band a site falls under, the ASPT score is plotted as a function of the SASS5 score (Dallas, 2007).

1.3 Aquatic invertebrate community metrics and lines of evidence in estuaries

To assess estuary ecosystem integrity, indices and statistical analyses are used. The indices used to determine ecosystem integrity include the: Water Quality Index (WQI); Benthic Index (BI); and Sediment Quality Index (SQI) (Borja *et al*. 2008). The analysis of biological and environmental factors will be further expanded upon in chapter 3. In South Africa there is no index that has been developed to assess estuarine system health using macrobenthic invertebrates as indicators of ecosystem health.

Statistical techniques are used to analyse and validate biological indices (Karr, 1999). The multivariate approach to analysing biological indices data looks at the relationship between aquatic invertebrates and the attributes of the habitats they have been collected in (Ollis *et al*., 2006). Different lines of evidence have been used in the absence of an index using macrobenthic invertebrates as indicators of estuarine ecosystem health. The lines of evidence used to evaluate estuarine macrobenthic invertebrates are species abundance, species richness and the Simpson's diversity index (Washington, 1984; Mohmad *et al*., 2015). To determine community assemblages of benthic macroinvertebrates, software packages such as PRIMER and Canoco are used for analyses (MacKay *et al*., 2010).

1.4 Lowland river and estuary land use change

Catchment water systems have maintained their integrity and functionality over time, however, human development has modified the landscape and that has an impact on the system (Karr, 1999). The lowland rivers and estuaries of KwaZulu-Natal are dynamic ecosystems that have high socio-ecological values (Begg, 1978; King and Pienaar, 2011). Threats associated with land

based activities are now known to threaten the ecosystem structure and integrity of many of these important ecosystems (Jewitt, 2002). If they are not managed effectively in a sustainable manner where a balance is reached between the use and protection of these ecosystems, the people and animals that depend on the wellbeing of the system will suffer. Multiple South African environmental protection and management acts (the South African Water Act, Coastal Management Act, National Environmental Management Act and Biodiversity act) state that a balance of use and protection of water bodies must be reached to manage these ecosystems for the future.

1.5 Aquatic invertebrates in the Amatikulu/Nyoni freshwater and estuarine system

The Amatikulu/Nyoni River and Estuary (29.05°S, 31.37°E) is a relatively small catchment (±60km in length) situated on the north coast of KwaZulu-Natal (Swemmer, 2009). This system is dominated by the extensive Amatikulu/Nyoni estuary which exceeds 12km of the Amatikulu and Nyoni Rivers. The river joins 4.5 km from the estuary mouth. The estuary is classified as a subtropical, open, barred, medium-large, type-F estuary (Whitfield, 2000; Harrison, 2004) and is partially protected by the Amatikulu Nature Reserve which includes the Nyoni section of the estuary and the South bank of the Amatikulu River. Although ecologically important the system is known to be threatened by agriculture activities, urban and peri-urban communities and industrial activities in particular (Carminati, 2008, Swemmer, 2009). Recent additional threats of climate change and droughts are also now known to threaten the wellbeing of the system.

1.6 Motivation and objectives

All around the world aquatic ecosystems should receive more attention due to their increased rate in degradation. By identifying the relationship between aquatic invertebrates with water quality and habitat, we gain insight into why aquatic ecosystems health has declined (Poulton *et al*. 2003). Very little is known about the Amatikulu/Nyoni catchment, this therefore advocates more research into the Amatikulu/Nyoni catchment's ecological wellbeing, to inform best management practices. Our study's main goal is to provide more detailed information about the catchment's water bodies and their current conditions. The overall aim of our study was to utilize aquatic macroinvertebrates as a tool to evaluate the current biological condition of the Amatikulu/Nyoni catchment. The objectives of the study are as follows:

1. To evaluate the response of freshwater macroinvertebrate community structure, abundance and distribution to water quality and other environmental variables from the Amatikulu River.
2. To determine how water quality and sediment composition affects estuarine benthic macroinvertebrates community structure, abundance and distribution.
3. To inform future best management practices for the Amatikulu/Nyoni catchment.

1.7 Thesis structure

The thesis consists of two data chapters (Chapters 2 and 3) which can be read and reviewed independently as they have been prepared for submission to international peer review journals. The research undertaken for each research chapter was conducted in the same lowland river catchment, therefore, some overlap could not be avoided.

Chapter 2. Response of Macroinvertebrates to water quality, quantity and habitat condition changes in the lowland Amatikulu River catchment, KwaZulu-Natal, South Africa.

Chapter 3. Response of macrobenthic invertebrates to water quality, quantity and habitat condition changes in the Amatikulu/Nyoni Estuary, KwaZulu-Natal, South Africa.

Chapter 4 is the overall conclusion which summerises and links the whole study. In this chapter sustainable management practice strategies were recommended to improve the ecological condition of the lowland Amatikulu/Nyoni Catchment.

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CHAPTER 2

Response of Macroinvertebrates to water quality, quantity and habitat condition changes in the lowland Amatikulu River catchment, KwaZulu-Natal, South Africa

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Abstract

Macroinvertebrates are one of the best organisms used as bioindicators to monitor river health in freshwater river systems. We carried out a study in the lowland Amatikulu River catchment with the aim to evaluate the current ecological health and drivers of the freshwater macroinvertebrate community assemblages. Here we used a rapid assessment approach known as the South African Scoring System version 5 (SASS5) and carried this out over two sampling seasons. The SASS score in comparison to the ASPT score and number of taxa was significant. The significant relationship between the biological community structure and the surveys conducted in the high flow and low flow seasons was expected because of seasonal variability. There was clear temporal variation, however it was mainly due to variability in taxa diversity between the two seasons. The availability of different biotopes played a role in the variability of the SASS score, with some taxa having a preference for certain habitat types especially sensitive taxa. The lowland Amatikulu River catchment sites showed that there was a change in the ecological condition of freshwater macroinvertebrate assemblages as well as along a longitudinal gradient with upstream sites having better conditions to support more sensitive species.

Key words: Macroinvertebrates, freshwater, SASS, low flow, high flow, river health.

2.1 Introduction

Water is essential for sustaining life and for the utilisation of the heavy and light industry, commercial farming and domestic use. Rivers in particular are not only important for human sustenance, they are also important for supporting aquatic organisms (Allan, 2004; Everall *et al*., 2017; Farrell *et al*., 2015). With an increase in water resource use, there are greater threats to the ecological status of freshwater systems (Roy *et al*., 2003; Shafie *et al*., 2017). Different land-use activities have different effects on river health which varies spatially and temporally (Miserendino *et al*., 2011; Everall *et al*., 2017). Activities such as agriculture; discharge of effluent; urban land-use; flow alteration; riparian clearing and sedimentation may alter the chemical and biotic conditions of the system (Allan, 2004; Farrell *et al*., 2015; Shafie *et al*., 2017). These activities may additionally alter the food source and habitat quality for river biota within the systems and this results in the degradation of the biotic community (Roy *et al*., 2003).

Water is an important resource in sub-Saharan Africa especially in drought prone countries like South Africa. South African surface freshwater ecosystems have become one of the most degraded in the world (Farrell *et al*., 2015). The degradation is due to the extensive utilisation of water resources in South Africa, hence there is a need for proper management and monitoring of these rivers to ensure that a balance between use and protection of these resources is maintained (RSA, 1998; Farrel *et al*., 2015).

Macroinvertebrates are a useful group of organisms to use as bioindicators for monitoring river health in freshwater river systems, especially since they are generally a good representation of the systems health whether good or bad and are found in many different habitats (Azrina *et*

al., 2006; Everall *et al*., 2017; Shafie *et al*., 2017). A high species diversity and presence of many intolerant species within the system during sampling indicates that the river health is in a good or acceptable ecological condition (Allan, 2004, Azrina *et al*., 2006). The presence or absence of taxa is also influenced by the state of environmental variables such as dissolved oxygen, temperature and organic content in the system (Hunte, 1978, Munn and Brusven, 1991). Macroinvertebrate distribution is also influenced by the types of habitat available such as vegetation and stones with some species as habitat specialists, furthermore the presence or absence of certain taxa is also affected by competition and predation (Wellborn *et al*., 1996).

The South African Scoring System (SASS) is a biomonitoring technique based on macroinvertebrate water quality tolerance, this information is used to determine the current ecological condition (EC) of a river system (Chutter, 1994, Dickens and Graham, 2002). It is now in its fifth version and is widely used in South Africa, this tool uses the attributes of macroinvertebrate communities and is the best scientific practice available (Dickens and Graham, 2002). In addition, multivariate statistical analysis is required to give a more in-depth look into the ecological integrity of a river or specific site so we used them for the study as additional lines of evidence as validation.

The lowland Amatikulu River system does not have any known information about its current ecological condition. Our aims in this study where we used the SASS5 tool to obtain information were (1) to determine which environmental variables drive the freshwater macroinvertebrate community assemblages at four freshwater sites within the lowland Amatikulu River catchment; (2) to investigate if there was any spatial or temporal variability in the freshwater macroinvertebrate community assemblages and; (3) to determine if there was a change in ecological condition down the longitudinal gradient of the lowland Amatikulu River

catchment. We predicted that the macroinvertebrate community assemblages would be affected by environmental variables.

2.2 Methods

2.2.1 Study site

Our study was carried out in the lowland Amatikulu River catchment (29° 4' 22.6596" S, 31° 33' 27.216" E) located on the north coast 103 km of Durban, South Africa (Figure 2.1). The lowland catchment is dominated by commercial sugarcane farming (Carminati, 2008). There has been relatively limited research conducted on macroinvertebrates within the lowland freshwater system with the Amatikulu River mainly being used as a reference site (Carminati, 2008). Downstream of the Amatikulu River (Below site AM7 and above site AM4, Figure 2.1) there is also the Hulett Amatikulu sugar mill located next to the Amatikulu River. Three of the Amatikulu River catchment sample sites (AM7, AM6 and AM4) have been previously used as part of the local industries and South African Pulp and Paper Industries (SAPPI) monitoring sites with AM5 added as a new sample site for this study (Figure 2.1).

The sampling site AM7 is characterized by sand and rocks with varying flows from low to fast. The sampling site is used by the community for washing clothes, drinking and for cattle, which were observed during sampling sessions. The river site has moderate sedimentation. The AM4 sampling site is characterised by sand and silt with slow to medium water flows. During sampling sessions, it was observed that the community and their cattle would cross through the river and the community members would also wash their vehicles. Informal settlements are present with the community reliant on the river site and an unmarked water extraction vehicle was active during both sampling sessions. Alien invasive plants dominated the riparian area and

there was limited habitat available, with no stones habitat for SASS. A high microbial load was observed at the AM4 site, possibly due to bovine agricultural waste. The AM6 sampling site was characterized by sand and medium water flows. There was extensive sedimentation. The site is used heavily by people in the surrounding settlements and there is extensive commercial sugarcane farming. Lastly the newly added AM5 sampling site was characterised by sand and gravel. The site was located under a bridge on the R102 road. Next to the site there are extensive commercial plantations. The site was characterised by very slow to sluggish water flow.

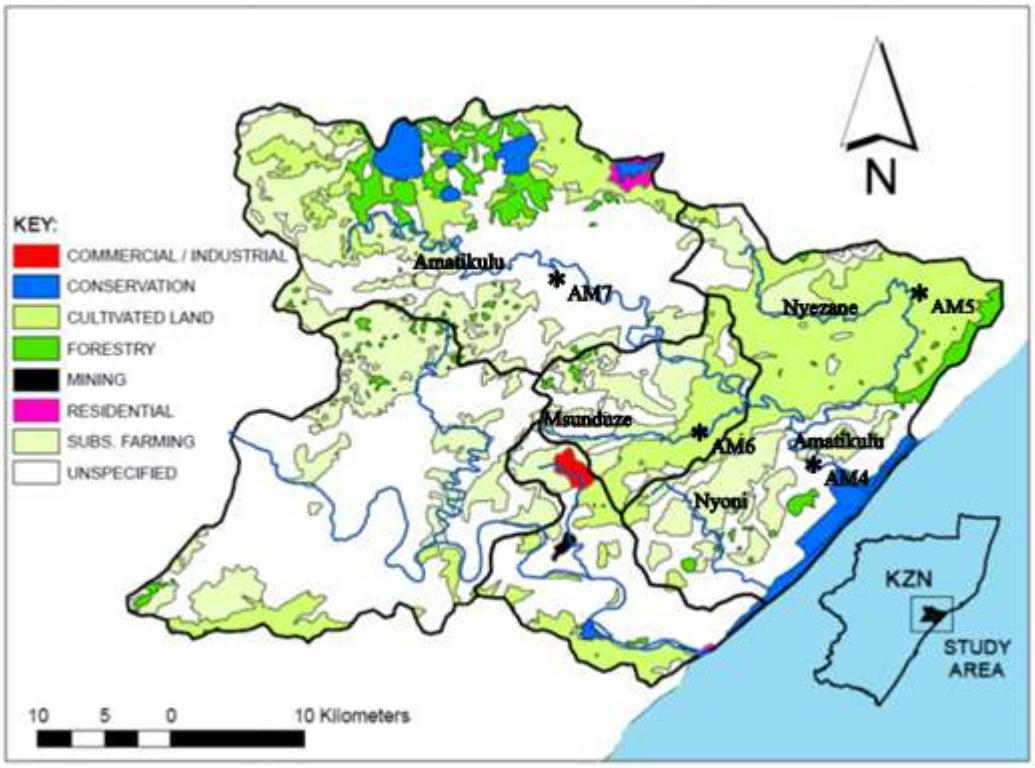

Figure 2.1 Map of the lowland Amatikulu River catchment freshwater sampling sites within KwaZulu-Natal, South Africa.

2.2.2 Water quality measurement

At each sampling site, we used a calibrated hand held YSI water quality meter (model 556 MDS, Yellow Springs, OH) to measure the following water variables: pH; dissolved oxygen (DO); temperature (^{o}C); electrical conductivity and salinity. Additionally, at each site a water sample was collected and stored in a polyethylene bottle (1 L) to analyse: chemical oxygen demand (COD); ammonia (NH3); nitrite (NO2), Chlorophyll a, nitrate (NO3); total phosphorus (TP); alkalinity; *Escherichia coli* (*E. coli*); coliforms; soluble reactive phosphorus (SRP); turbidity; fluoride (F) and calcium (Ca). The polyethylene bottles were stored in the refrigerator to preserve and prevent further change in the water by metabolism of organisms before reaching the laboratory for analysis (Azrina *et al*., 2006) at Umgeni Water (Pietermaritzburg, South Africa).

2.2.3 Biological sampling

We collected biological samples for this study using SASS5. The sampling was executed according to the methods of Dickens and Graham (2002). For each sampling site (AM4, AM5, AM6 and AM7) three biotopes were sampled where available. The biotopes included the vegetation, GSM (gravel, sand and mud) and stones biotopes, which were sampled using a standard kick net (30 cm x 30 cm) with a mesh size of 1 mm. The stones habitat was further divided into stones in current (SIC); stones out of current (SOOC); and bedrock. In the stones biotope, the standard kick net was placed firmly on the river bed and the net was positioned so that the water flows into the net entrance, the collector would then kick and shuffle the stones in front of the net to dislodge the macroinvertebrates. The SIC and bedrock habitats were sampled for 2 to 5 min. max. The SOOC habitat was sampled for 1 min. The vegetation biotope was also further divided into aquatic vegetation; marginal vegetation out of current (OOC); and marginal

vegetation in current (IC). To collect the macroinvertebrates, the kick net is swept in the IC and OOC for 2 min. in total and 1 min. in the aquatic vegetation. For the GSM habitat, where there is gravel, sand and mud, the area was stirred up by stomping and twisting in place then sweeping the net through the GSM plume for 1 min. For an additional 1 min. the collector handpicked individual organisms and made visual observations and record in the biotope where found by circling the estimated abundance on the SASS5 score sheet.

After we collected samples from the available biotopes, they were poured into their individual respective white plastic trays (45 cm x 30 cm x 8 cm) and the macroinvertebrates were sorted, identified (maximum 15 min. using the standard SASS5 identification manual) and scored with estimated abundances (1 = 1; A = 2-10; B = 10-100; C = 100-1000; and D = >1000) on the standard SASS5 sheet according to SASS5 protocol. The samples were then stored into plastic honey jars and 70% ethanol was added into the jars for preservation. Samples were sorted and individual organisms later counted in the laboratory (Lenat and Barbour, 1994; Resh *et al*., 1995). Using a handheld soil scoop, an additional 1 kg sediment sample was collected at each site to evaluate grain size distribution.

2.2.4 Sediment analysis

Initially the 1 kg sediment samples were dried at 60 ^{o}C in an oven for 24 h to remove moisture (Shaddock and Wepener, 2015). We determined grain size distribution with a standard coarse and fine aggregate sieve analysis. A mechanical shaker was used for the aggregate test with sieve mesh sizes ranging from 750 mm to 0.053 mm. The data for each sampling session and site were inputted into an Excel spreadsheet and further sorted into coarse, medium and fine grain sizes.

2.2.5 Data analyses

To determine taxa diversity for each site, we calculated the Shannon Weiner Diversity Index (Washington, 1984) in Excel. Ordination techniques were performed using the Canoco (version 4.5 software) on the macroinvertebrate abundance data to explore differences between sampling sites and the potential influence of water quality variables responsible for the respective macroinvertebrate community assemblages (Van den Brink *et al*., 2003). Redundancy analysis (RDA) were undertaken on the data sets using Canoco (version 4.5 software) and with the availability of macroinvertebrate abundance data, data were transformed using Log X+2 transformation (Van den Brink *et al*., 2003). SASS scores were used to determine ecological condition using established biological bands and categorised accordingly (Table 2.1) (Dallas, 2007).

Table 2.1 The ecological classes of sites determined from ASPT as a function of the SASS5 score (adapted from Dallas, 2007).

Biological band	Condition	Description	Colour
A	Natural	Unmodified or natural	Blue
B	Good	Largely natural with few modifications	Green
C	Fair	Moderately modified	Yellow
D	Poor	Largely modified	Red
E	Seriously modified	Seriously modified	Purple
F	Critically modified	Critically or extremely modified	Black

2.3 Results

Our collection of 24 samples from the eight sites during the respective high flow and low flow sampling seasons yielded 30 macroinvertebrate families. These comprised of 867 individuals for the three biotopes upon which the assessments were based. The sampling sites AM6 and AM4 had a lower diversity in comparison with the other two sites (Figure 2.2). There was no distinct seasonal trend between sampling seasons, the AM7 site had the highest diversity during the low flow sampling period whereas AM5 had the highest diversity during the high flow season. The AM4 site had the poorest macroinvertebrate community assemblages in comparison to the other sites (Table 2.2). The water quality variables recorded during the low and high flow sampling period fall within DWAF target water quality guidelines (Table 2.3).

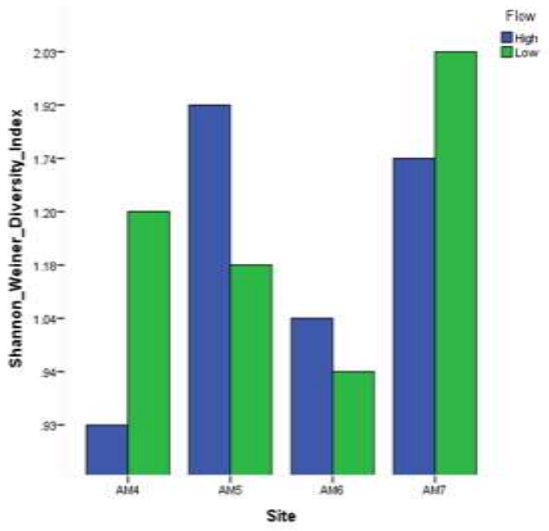

Figure 2.2 Graphical representation of the Shannon Weiner Diversity Index during the high and low flow sampling period at the Amatikulu River sites.

Table 2.2 Environmental variables recorded for the high and low flow sampling period at each of the Amatikulu River sites in 2017. The H and L suffix indicate the sites during the high and low flow respectively.

		Site							
Environmental variable	**Units**	**AM4_LF**	**AM6_LF**	**AM7_LF**	**AM5_LF**	**AM4_HF**	**AM6_HF**	**AM7_HF**	**AM5_HF**
Alkalinity	Ppm	170	114	16.1	101	80.2	24.9	18.5	88.3
Chloride	mg/L	105	199	43.2	242	98.3	60.5	50.7	211
NO2	mg/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
NO3	mg/L	<0.10	<0.10	0.59	<0.10	<0.10	<0.10	<0.10	<0.10
SO4	mg/L	22.3	16.6	4.34	34.3	10.9	<1.00	4.38	44.4
Ca	mg/L	26.4	25.1	3.90	28.3	14.7	7.17	4.46	21.0
Chlorophyll a	mg/L	3.26	2.36	<0.14	3.97	2.08	9.69	1.28	2.46
COD	mg/L	<20	<20	<20	22	29	35	24	<20
Coliforms	counts/100mL	>2420	>2420	>2420	>2420	>2420	1120	>2420	4839
E.coli	counts/100mL	155	172	16	488	114	11	6	37
EC	mS/m	58.0	94.8	19.2	111	50.4	26.0	22.6	100
F	mg/L	183	164	<100	171	148	<100	123	208
HPC 37		>1000	>1000	>1000	>1000	>1000	220	>1000	>1000
Na	mg/L	93.0	87.5	27.5	141	62.2	36.7	27.2	137
NH3	mg/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
SRP	mg/L	8.47	<5.00	8.15	6.42	15.8	12.1	20.3	13.4
TP	mg/L	79.8	164	23.8	67.0	35.1	28.1	33.0	56.7
Turbidity	NTU	14.4	9.5	3.7	13.9	11.8	8.0	4.0	17.6
Temperature	ºC	22,43	19,01	16,85	17,94	24,8	20,8	18,7	16,5
Ph		7,02	6,67	5,01	7,76	7	6,27	7,1	5,96
Dissolved oxygen	mg/L	9,93	9,86	25,53	9,01	7,45	7,12	8,79	9,65
Dissolved oxygen	%	114,7	106,6	268,7	95,4	88,8	82,7	98,3	
Course sediment	%	68,11	53,60	10,29	47,0	68,11	53,60	10,29	47,09
Fine sediment	%	31,02	45,94	85,44	60,66	31,02	45,94	85,44	60,66
Very fine sediment	%	0,88	0,46	4,27	0,91	0,88	0,50	4,27	0,91

Table 2.3 Benthic macroinvertebrates abundances collected at the Amatikulu River sites during the high and low flow sampling period in 2017. The H and L suffix indicate the sites during the high and low flow respectively.

	Sites							
Taxa	**AM7_H**	**AM6_H**	**AM4_H**	**AM5_H**	**AM7_L**	**AM6_L**	**AM4_L**	**AM5_L**
Amphipoda (Scuds)	8	14		3				
Potamonautidae* (Crabs)	3				1			
Atyidae (Freshwater shrimps)	22	29	18	2	11	21	7	37
Baetidae 1sp					5			
Baetidae 2 sp	5	53		17	12	17	13	16
Baetidae > 2 sp	71	23	31					
Caenidae (Squaregills/Cainfles)	1	9						
Coenagrionidae (Sprites and blues)		6			3			
Aeshnidae (Hawkers & emperors)								1
Corduliidae (Cruisers)							1	
Gomphidae (Clubtails)	3						1	
Libellulidae (Darters/skimmers)		1		1	3		1	
Belostomatidae* (Giant water bugs)		1	2					
Corixidae* (Water boatmen)		3						
Naucoridae* (Creeping water bugs)				4				
Veliidae/M...veliidae* (Ripple bugs)	8	2			15			
Hydropsychidae 1 sp	1							
Hydropsychidae 2 sp	14							
Hydroptilidae		2						
Leptoceridae						1		8
Dytiscidae/Noteridae* (Diving beetles)						1		
Elmidae/Dryopidae* (Riffle beetles)		3						
Gyrinidae* (Whirligig beetles)					11			
Ceratopogonidae (Biting midges)		6	2					1
Chironomidae (Midges)	17	48	36	10	14	7		13
Simuliidae (Blackflies)					5			28
Ancylidae (Limpets)				38				
Thiaridae* (=Melanidae)			60	16			19	
Corbiculidae (Clams)			1					

The preliminary RDA analysis with all the environmental variables and Monte Carlo permutation tests indicated that there was no significant effect of environmental variables on the macroinvertebrate community structure. This was mainly due to a high collinearity of environmental variables. To determine which environmental variables had a significant impact on the species abundance data, we carried out an RDA analysis again with forward selection (automatic and manual) of variables using Canoco (version 4.5).

The first run considered the potential relationship between the biological community structure and the sampling sites (Figure 2.3a). The test showed that the relationship was not significant ($p = 0.549$), however, certain species such as the more sensitive Trichoptera (caddisflies) and Ephemoptera (mayflies) groups were consistently clustered around AM6 and AM7. The ordination plot also showed that AM4 was the most dissimilar sampling site compared with the other three sampling sites. The second run was to examine the relationship between biological community structure and the respective surveys conducted in high flow and low flow (Figure 2.3b). There was a significant relationship ($p = 0.044$) with more taxa clustered around the high flow season in the ordination plot with more sensitive taxa such as Amphipoda present (Figure 2.3b).

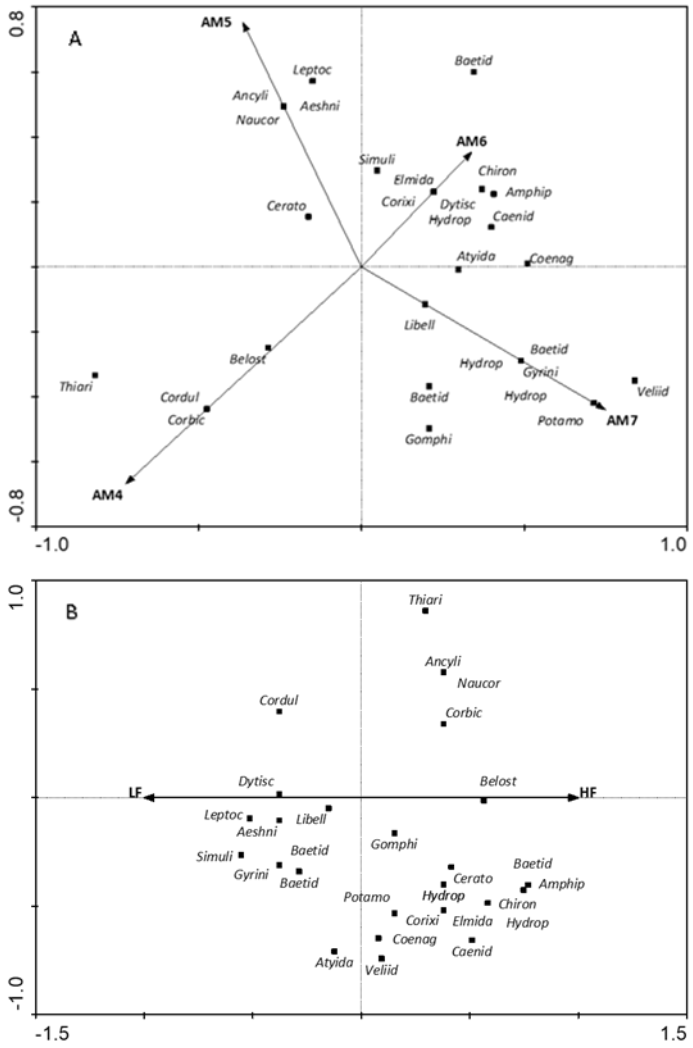

Figure 2.3 RDA ordination of freshwater macroinvertebrate community assemblages of the Amatikulu River sites in relation to (a) the sampling sites (variance on the 1st axis is 50.4 % and an additional 29.1 % on the 2nd axis) and (b) the sampling survey seasons (variance on the 1st axis is 23.3 % and an additional 28 % on the 2nd axis) with HF and LF are the suffixes for high flow and low flow respectively.

The third run was to determine whether there was a relationship between biological community structure and SASS outcomes (ASPT, number of taxa and SASS score) (Figure

2.4a). When all three SASS variables were tested at the same time to determine if there was a relationship with the freshwater biota, there was no significance ($p = 0.220$). Through manual selection, the SASS score in comparison to the other two variables was significant ($p = 0.012$). The fourth run was to evaluate the relationship between the biological community structure and all the water quality variables combined (Figure 2.4b). There was no significant relationship ($p = 1.000$), however, the variables *E. coli* ($p = 0.08$); chloride ($p = 0.09$) and dissolved oxygen saturation ($p = 0.075$) displayed high correlation that may have had an important influence on the freshwater biota communities. The majority of taxa cluster around dissolved oxygen. The ecological condition during the high flow season ranged from a B (largely natural with few modifications) to D (largely modified) (Figure 2.5 and Table 2.4). During the low flow season, the ecological condition of the sites ranged from C (moderately modified) to D during low flow. The AM4 site improved from D to C whereas AM7 deteriorated from B to C showing temporal fluctuations in water quality.

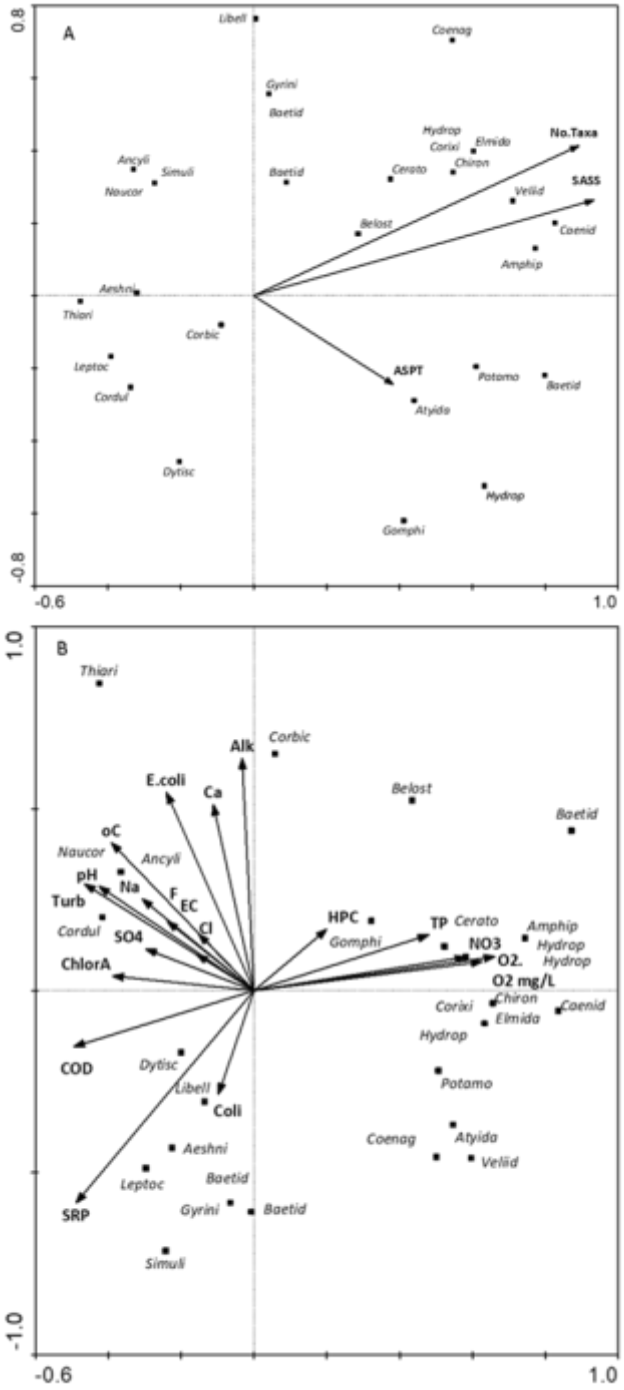

Figure 2.4 RDA ordination of freshwater macroinvertebrate community assemblages of the Amatikulu River sites in relation to (a) the SASS5 variables (variance on the 1st axis is 63.8 %

and an additional 24.2 % on the 2nd axis) and (b) the water quality variables (variance on the 1st axis is 31 % and an additional 23.9 % on the 2nd axis).

Table 2.4 SASS5 results of the Amatikulu River sites showing the SASS5 score, number of taxa and ASPT score to determine the ecological condition of each site during the high and low flow periods.

Site	Ecoregion	Flow	SASS 5 Score	No. of Taxa	ASPT	Biological band
AM7	Northern eastern uplands	High	71	11	6.45	B
		Low	54	10	5.4	C
AM6	Northern eastern coastal belt	High	85	14	6.07	C
		Low	27	5	5.4	D
AM5	Northern eastern coastal belt	High	35	8	5	D
		Low	40	7	5.71	D
AM4	Northern eastern coastal belt	High	38	7	5.53	D
		Low	35	6	5.8	C

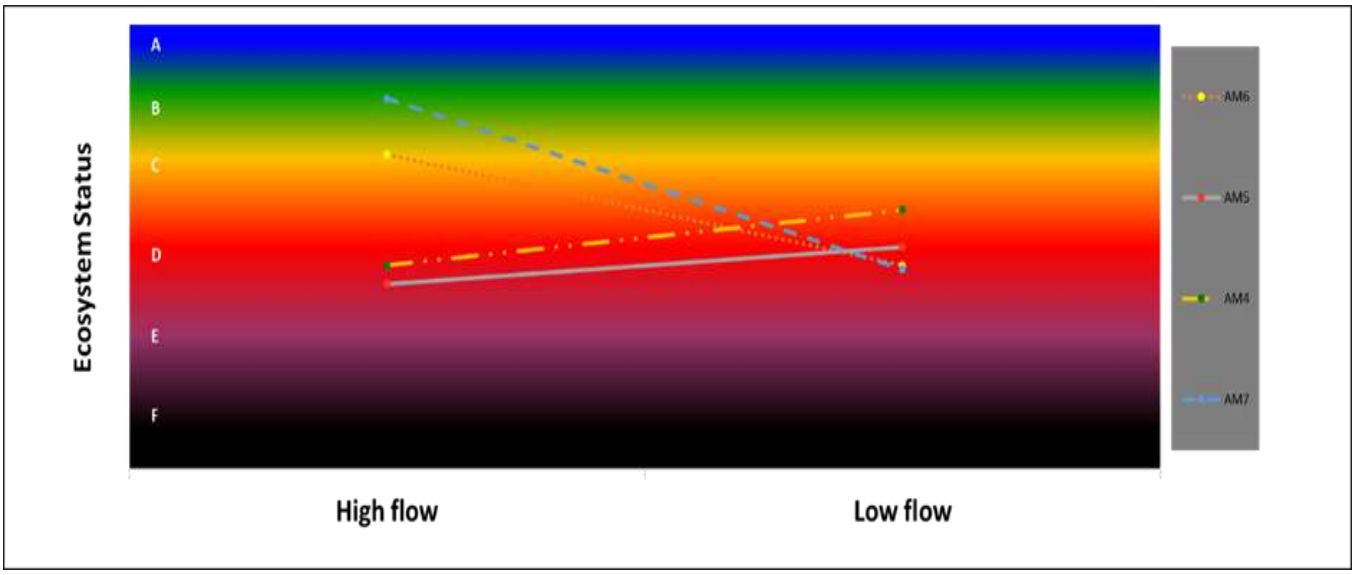

Figure 2.5 Trend assessment of the wellbeing of the Amatikulu lowland river catchment sites during the high- and low flow seasons.

The fifth run was to evaluate whether there was a significant relationship between the biological community structure and sediment composition (Figure 2.6). There was no significance ($p = 0.476$) in the relationship between the freshwater biota and sediments, however, there were species that clustered around specific grain sizes and sediment grains sizes varied between the two sampling seasons (Figure 2.6 and Table 2.3).

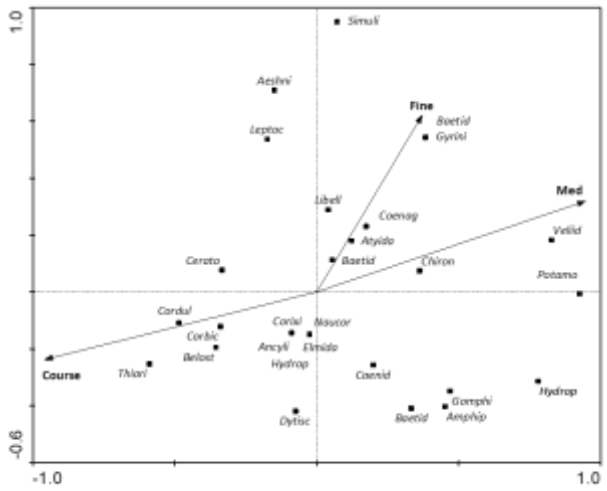

Figure 2.6 RDA ordination of freshwater macroinvertebrate community assemblages of the Amatikulu River sites in relation to the sediment grain size. Variance on the 1st axis is 42.1 % and an additional 34.6 % on the 2nd axis.

2.4 Discussion

As predicted, we found that the macroinvertebrate community assemblages in the Amatikulu River were affected by environmental factors, natural and anthropogenic. The lower portion of the river (AM4) had the poorest macroinvertebrate community assemblages compared with the other three sampling sites. This can be attributed to constant water abstraction and sand mining which alter the biota's habitat and remove habitats such as vegetation (Matthaei *et al*., 2010; pers. obs.). Due to the activities mentioned above, there was a loss of vegetation which was observed at the site during the study. The Chironomidae taxon was present and abundant at all the sampling sites which may be due to higher organic pollution represented by relatively high phosphorus and nitrogen levels (e.g. untreated faecal matter) which the taxon prefers (Munn and Brusven, 1991, Bunn and Arthington, 2002). Moreover, Chironomidae is a relatively resilient

taxon. Atyidae is also one of the dominant taxa present in the groups. The Atyidae taxon is known to be abundant in habitats that have relatively stable conditions (Hunte, 1978), which may indicate there are stable conditions at the sampling sites.

There was no significance in the relationship between sampling sites and the freshwater macroinvertebrate community, however the presence of some pollution indicators in the upper reaches (AM6 and AM7) above intensive anthropogenic activities (such as commercial sugarcane farming) may indicate negative impacts (e.g. increasing organic nutrients) on the river ecosystems (Nhiwatiwa *et al*., 2017). The upper reaches (AM6 and AM7) were the only sampling sites with intolerant taxa (Trichoptera and Ephemoptera). This is indicative of low pollution, better water quality and greater habitat availability in comparison with the other two sampling sites further downstream (Poff *et al*., 1997, Allan, 2004, Azrina *et al*., 2006). The sites with intolerant taxa present were also more isolated mainly with human settlements and livestock farming activity.

Water flow affects habitat availability, which also influences the freshwater macroinvertebrate community abundance, diversity and distribution (Bunn and Arthington, 2002, Resh *et al*., 1988). The significant relationship between the biological community structure and the surveys conducted in the respective high flow and low flow seasons was expected as seasonal variability influences habitat, which is an important driver in species communities (Beche *et al*., 2006). There was clear temporal variation, however, it was mainly due to variability between the two seasons. During low flow, water quantity and quality generally decreased; therefore, increasing habitat loss and poorer water quality conditions, thus supporting fewer taxa at lower densities (Beche *et al*., 2006, Bunn and Arthington, 2002, Leunda *et al*., 2009). The significant relationship between freshwater biota and the surveys can also be attributed to the natural

seasonal variability which in other studies of lowland river systems has been documented to significantly vary just like in our study (Leunda *et al*., 2009, Šporka *et al*., 2006).

The SASS5 outcomes have been widely used in South Africa to assess water quality and varying degrees of pollution. Multiple studies in rivers demonstrated how the SASS score is a good indicator of water quality (Armitage *et al*., 1983, Harrison and Elsworth, 1958, Dallas, 1997, Gratwicke, 1998). However, the availability of different biotopes plays a large role in the variability of the SASS score, some taxa have a preference for certain habitat types, especially sensitive taxa (e.g. Ephemeroptera) (Gratwicke, 1998). Seasonal variation of water quality also influences the SASS score temporally which is also a significant driver of freshwater macroinvertebrate communities (Dallas, 1997). Biotopes are known to be home to distinct species, which is also an indication of habitat preference. During low flows, habitats are reduced or lost, therefore fewer species will be present. During the high flow season, there are relatively more taxa present than in the low flow season and this has been documented by other researchers (Bunn and Arthington, 2002), which can explain why the SASS scores varied temporally in our study.

Water quality variables play an important role in the ecosystems biota survival and prevalence. Most of the environmental constituents recorded for the two surveys fall within target water quality guidelines (DWAF, 1996). Dissolved oxygen is one of the most important water quality constituents, because it can also influence species diversity, distribution and abundance (Harrison, 2016). The data showed that two upper reach sites (AM7 and AM6) and one lower reach site (AM4) had oxygen saturation levels that exceeded 100% during high flow; while one lower reach site (AM5) had oxygen saturation levels that exceeded 100% during low flow. The elevated oxygen levels were referred to as super saturation by Dallas and Day (2004).

Super saturation in in parts of rivers where there are no waterfalls is often caused by water stagnation (no flow) or sluggish water flow (Dallas and Day, 2004). *High Escherichia coli* (*E. coli*) abundances were present at all sites suggesting high nutrient loads from livestock and organic domestic wastes from surrounding communities. Chironomidae were present at all the sites but at some sites with higher abundances which indicates variability in organic pollution which the taxon prefers (Munn and Brusven, 1991, Bunn and Arthington, 2002).

Sediment grain size distribution had no significant relationship with the freshwater macroinvertebrate community due to very little change in grain sizes over the two sampling seasons. This showed that the grain size distribution was consistent, and the freshwater macroinvertebrate community associated with this habitat did not change significantly between sites and surveys. Only two surveys were conducted for this study, therefore there was a limited amount of data to evaluate a trend and that may have been the reason for no change. In this study there were taxa such as Thiaridae which showed preference for the stones habitat; and in South Africa, Thiaridae generally prefers both the stones and GSM habitat (De Kock and Wolmarans, 2009).

Using the ASPT as a point of SASS5 outcomes, the ecological condition of each sampling site was determined during the different seasons using the established biological bands (Dallas, 2007). The overall results for these sites suggested that although the AM7 (upstream) was moderately modified, land-use activities are affecting the wellbeing of the other sites further downstream. The decline in the ecological condition of upper sites (AM6 and AM7) may be attributed to seasonal variation in habitat and water quality (Bunn and Arthington, 2002, Dewson *et al*., 2007). The lower sites (AM4 and AM5) showed recovery which can also be attributed to more habitat availability, water quality and less disturbance.

Our study on the lowland Amatikulu River catchment's ecological status showed that the sites that were sampled were in a degraded state in both high and low flow sampling seasons. Due to the low ecological statuses of the river sampling sites, the sites need immediate attention to prevent the sites status from further deteriorating. The most persistent problem was the increased nutrient loads resulting from the surrounding areas and land-use activities. The study was able to provide an idea as to what are the potential drivers of the macroinvertebrate community and the condition of the lowland Amatikulu River catchment with the use of the SASS5 tool. The lowland Amatikulu River catchment sampling sites showed that there was a change in ecological condition of the freshwater macroinvertebrate assemblages especially along a longitudinal gradient with upstream sites having better conditions to support more intolerant species.

2.5 Acknowledgements

We are grateful to the Aquatics Ecosystems Research (AER) group for assistance in the field. We thank the National Research Foundation (ZA); Ford Wildlife Foundation, AER and the South African Pulp and Paper Industries (SAPPI) for financial support.

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CHAPTER 3

Response of macrobenthic invertebrates to water quality, quantity and habitat condition changes in the Amatikulu/Nyoni Estuary, KwaZulu-Natal, South Africa

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Abstract

Estuaries are very complex and dynamic ecosystems which host a large diversity of biota. Monitoring of the ecosystem condition is important for informing management practices. We investigated the macrobenthic community structure and distribution in the Amatikulu/Nyoni Estuary and assessed how their structure and distribution related to the surrounding environmental factors. To collect benthic sediment samples a Van Veen grab was used. During each visit three replicate samples were collected from each site and individual organisms were later identified in the laboratory. There was a significant relationship between the estuarine macrobenthic community assemblages and the sampling surveys which was expected with more species cluster around survey 2 when the estuary mouth was closed. It has been documented that when the mouth is closed in a temporary open/closed estuary, the habitat is relatively stable; water levels are higher; and more species are available due to favourable conditions. The Amatikulu/Nyoni Estuary supported a variety of macrobenthic communities. However, more

research is needed over an extended period of time to determine how these macrobenthic communities change with time.

Key words: Estuary; macrobenthic community; flood; mouth permanence; temporary open/closed estuary.

3.1 Introduction

Estuaries are an environment where freshwater and seawater interact (Breen and McKenzie, 2001). Estuaries are dynamic ecosystems which host a large diversity of biota and they are considered among the most socio-ecologically important ecosystems (Begg, 1984; Kalejta and Hockey, 1991; Herman *et al*., 1999; Dauer *et al*, 2000; Carvalho *et al*., 2005; Cardoso *et al*., 2008; Whitfield *et al*., 2008; Adams 2014; Dittman *et al*., 2015). Estuarine ecosystems provide multiple services such as trapping nutrients; natural fisheries; and filter toxic pollutants (Adams, 2014). When intact, estuaries are good tourist attractions and they have educational and cultural value (Cardoso *et al*., 2008; Adams, 2014). These ecosystems provide varying habitats for fauna and flora such as fish at different life stages as well as macroinvertebrates (Adams, 2014). Each of these species plays a role in the ecosystems functionality, and they are also affected by anthropogenic or natural activities (Herman *et al*., 1999). The spatial and temporal variations that occur in and around estuarine ecosystems influence their macrobenthic community structures, resulting in the alteration of their ecosystem functionality (Nozais *et al*., 2005; Ortega-Cisneros *et al*., 2016). Due to increased anthropogenic land-use activities and natural occurrences such as drought and floods, estuarine ecosystems are under regular stress and this affects the fauna, particularly by altering available habitat and resources (Cardoso *et al*., 2008). Anthropogenic

land-use activities such as agriculture and heavy/light industries which extract resources from the estuaries or deposit nutrients and chemical discharge into the system cause sedimentation, nutrient loading, and water contamination (Dauer *et al*, 2000).

The physical and chemical state of estuaries is also greatly affected by the permanence of the estuary mouth, tidal action and freshwater inflow (Dix *et al*., 2008; Hanekom and Russell, 2015; Ortega-Cisneros *et al*., 2016). Macrobenthic invertebrate assemblages have played an important role as indicators to address environmental issues such as pollution in estuaries (Gaston *et al*. 1999). Estuaries vary in their chemical and physical constituencies due to natural and anthropogenic processes (Gaston *et al*. 1999; Dix *et al*., 2008). The alteration of the chemical composition of estuaries affects the macrobenthic communities structure and abundance (Gaston *et al*., 1999). Macrobenthic invertebrate assemblages in estuaries closer to anthropogenic development or activities tend to have lower species richness than in areas further away from these activities (Gaston *et al*., 1999).

South Africa has a coastline of 3 100 km with 250 recognised functioning estuaries (Turpie *et al*., 2002; Hanekom and Russell, 2015). The majority of the South African population is concentrated on the coastline; therefore, the ecological integrity of many South African estuaries has been threatened (Begg, 1978, Turpie *et al*., 2002). Due to the threats that South African estuaries are facing as result of anthropogenic activities, they have become functionally degraded, resulting in the loss of species and ecosystem processes (Turpie *et al*., 2002). An understanding of estuarine systems has become a requirement to manage and maintain them. The Amatikulu/Nyoni Estuary is an ecosystem that has had various studies performed on its different aspects. The Amatikulu Estuary has had studies conducted on its morphology and sedimentology

(Begg, 1978) and on the fauna within the estuary, mostly on fish (O'Brien *et al*., 2009). There is no published information pertaining to the macrobenthic invertebrate community structure and their drivers. We investigated the macrobenthic community structure and distribution in the Amatikulu/Nyoni Estuary and determined how their structure and distribution related to the surrounding environmental factors. We predicted that the main drivers of the macrobenthic invertebrate community assemblage structure and composition would be the sediment grain size distribution and salinity.

3.2 Materials and Methods

3.2.1 Study site

Our study was conducted at the Amatikulu/Nyoni Estuary located 105 km north of Durban in the South African province of KwaZulu-Natal and is part of the Amatikulu Nature Reserve (29^{o} 05’ S; 31^{o} 38’ E). The estuary is classified as a temporary open/closed estuary (TOCE) (Begg, 1978; Ferreira, 2010). The Amatikulu Estuary covers an area of 122 ha (Begg 1978). The estuary is fed by the Amatikulu and Nyoni Rivers. The Amatikulu Estuary’s mouth barrier is breached during floods (Begg, 1978), Cyclone Dineo in 2017 caused a breach in the barrier leaving the Amatikulu mouth open. Samples were collected twice during late May/early June (Autumn) and late August 2017 (Spring). During the 1st survey the mouth was still open due to heavy floods from Cyclone Dineo which resulted in relatively low water levels in the estuary, but the estuary mouth was closed during the 2nd survey. Due to the heavy floods resulting from Cyclone Dineo, the organic matter that had accumulated on the estuary bed after 2 years of drought was washed away leaving small remnants of vegetation and roots (pers. obs.). The estuary sites are existing

sites for the estuary monitoring programme under the South African Department of Water and Sanitation (DWS). The sites AM2, AM3 and NE1 are situated in the upper reaches of the estuary dominated by fine to very fine sediments (clay and silt), whereas AM1, situated at the mouth is dominated by coarse (sand) sediments (Figure 3.1). The deepest area of the estuary is about 7 m deep close to AM2 with the shallowest area about 0.15 m.

Figure 3.1 Map showing locations of the sampling sites on the Amatikulu/Nyoni Estuary, South Africa.

3.2.2 Water quality measurement

At each sampling site, we used a calibrated hand held YSI water quality meter (model 556 MDS, Yellow Springs, OH) to measure the following: pH; dissolved oxygen (DO); temperature (^{o}C);

electrical conductivity and salinity. Additionally at each site a water sample was collected and stored in a polyethylene bottle (1 L) to analyse: chemical oxygen demand (COD); ammonia (NH3); nitrite (NO2), Chlorophyll a, nitrate (NO3); total phosphorus (TP); alkalinity; *Escherichia coli* (*E. coli*); coliforms; soluble reactive phosphorus (SRP); turbidity; fluoride (F) and calcium (Ca).The polyethylene bottles were stored in the refrigerator to prevent change in the water by metabolism of organisms (Azrina *et al*., 2006) and then taken to Umgeni Water (Pietermaritzburg, South Africa) to be analysed in their laboratories to determine the physiochemical constituents in the water.

3.2.3 Biological data collection

We used a rapid assessment approach for the collection of macrobenthic invertebrates at four estuary sites (AM1, AM2, AM3, and NE1) in the Amatikulu/Nyoni Estuary. To collect benthic sediment samples a Van Veen grab (0.024 m^2 bite, Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) which samples a depth of 15.5 cm was used. During each visit three replicate samples (five grabs per replicate sample) were collected using the grab and decanted into 10 L buckets, 10 ml of 20 % formaldehyde was added to the replicate sample and stirred to shock the benthic invertebrates out of the sediment. Each sample was washed through a 500 µm sieve to remove excess material, the remaining animals and debris were preserved in honey jars (500 ml) containing a 10% formaldehyde solution. Rose Bengal dye was added to each preserved replicate sample in the field to aid in sorting (Palmer *et al*., 2016) and counting in the laboratory. Samples were identified to furthest taxonomic resolution. An additional grab sample at each site was collected for sediment analysis. Samples were dried at 60 ^{o}C in an oven for 24 h to remove

moisture (Ortega-Cisneros *et al*., 2017) so that it does not bubble over and contaminate the other samples. Using the sediment samples, the particle size distribution was determined using a course and fine aggregate sieve analysis. A mechanical shaker was used for the aggregate test with sieve mesh sizes ranging from 750 mm to 0.053 mm and the material retained in each mesh sieve was recorded. Total organic carbon (TOC) was determined using the loss on ignition (LOI) method whereby samples were dried at 60 ºC for 24 h and then placed in a furnace at 600 ºC for 6 h (Shaddock and Wepener, 2015). The data for each sampling session and site were inputted into an excel and further sorted into course, medium and fine grain sizes.

3.2.4 Data analyses

We sorted sediment grab samples to remove microbenthic invertebrates to be identified in a laboratory at the University of KwaZulu-Natal (Pietermaritzburg campus) using a microscope (Leica ZOOM 2000, Buffalo, NY). Specimens were identified to the furthest taxonomic resolution using different taxonomic guides (Day, 1969, Griffiths, 1976, Kensley, 1978) and numbers of individual specimens for each taxon captured in an Excel spreadsheet for each sampling site for both sampling seasons.

We calculated taxon diversity for each of the sites using the Shannon Weiner Diversity Index (Mohmad *et al*., 2015) in Excel. A Redundancy analysis (RDA) was performed using Canoco version 4.5 software to evaluate the drivers of the estuarine macrobenthic community within each site in the Amatikulu/Nyoni Estuary and the influence of environmental factors on the community assemblages. Data were transformed using Log X+2 transformation (Van den Brink *et al*., 2003).

3.3 Results

We identified a variety of estuarine macrobenthic invertebrates in the Amatikulu/Nyoni Estuary in 2017. A total of 24 samples were collected which yielded 21 taxonomic groups with 2369 individuals. The taxa identified for this study included: Polychaeta (42.5 %); Amphipoda (24.6 %); Cumacea (12.4 %); Isopoda (9.5 %); Gastropoda (5.2 %); Copepod (2.7 %); Diptera (2.3 %); Decapoda (1.4 %); Bivalvia (0.08 %); and Tanaidacea (0.04 %) (Table 3.1). There was a higher taxa abundance and diversity during the 2nd survey in comparison with the 1st survey (Table 3.1, Figure 3.2).

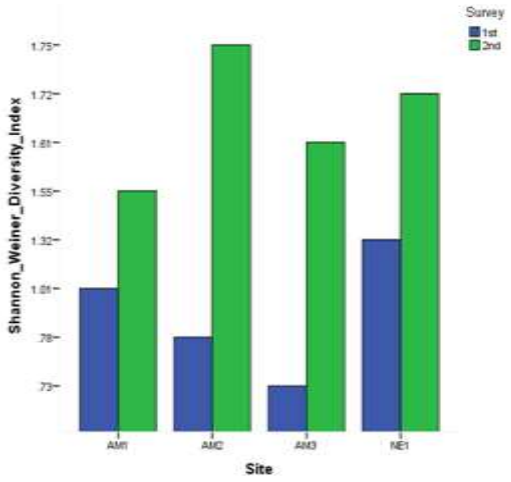

Figure 3.2 Estuarine macrobenthic species diversity in the Amatikulu/Nyoni Estuary over two sampling sessions.

Each site had a higher taxa diversity during the 2nd sampling session when the estuary mouth was closed, however, each site had a lower taxa diversity during the first sampling session when the mouth was open after heavy floods (Figure 3.2). Neriedidae (Polychaeta) was highly

abundant at all the sites during both sampling sessions. Direct analyses (RDA) were undertaken on the data sets, additionally data was transformed using Log X+2 transformation (Van den Brink *et al*., 2003). The first attempt at the RDA analysis with all the environmental variables and Monte Carlo permutation tests indicated that there was no significant effect of environmental variables on the macroinvertebrate community structure in the Amatikulu/Nyoni Estuary sites. This was mainly due to high collinearity of environmental variables. To determine which environmental variables had a significant impact on the species abundance data, an RDA analysis was carried out again with forward selection (automatic and manual) of variables.

The Amatikulu/Nyoni Estuary sites did not have a relationship with the estuarine macrobenthic community assemblages (Figure 3.3a; $p = 0.708$). The ordination plot indicated that the mouth site (AM1) was the most dissimilar site to the others and that the other inner sites were more similar to each other with having higher diversity and abundance of macrobenthic invertebrates (Figure 3.3a). The second run of statistical analysis to evaluate if there was a relationship between the estuarine macrobenthic community assemblages and the sampling surveys, there was a relationship (Figure 3.3b; p-value = 0.023). More species were clustered around survey 2 when the mouth was closed in comparison with survey 1 when the mouth was open due to heavy floods from Cyclone Dineo (Figure 3.3b).

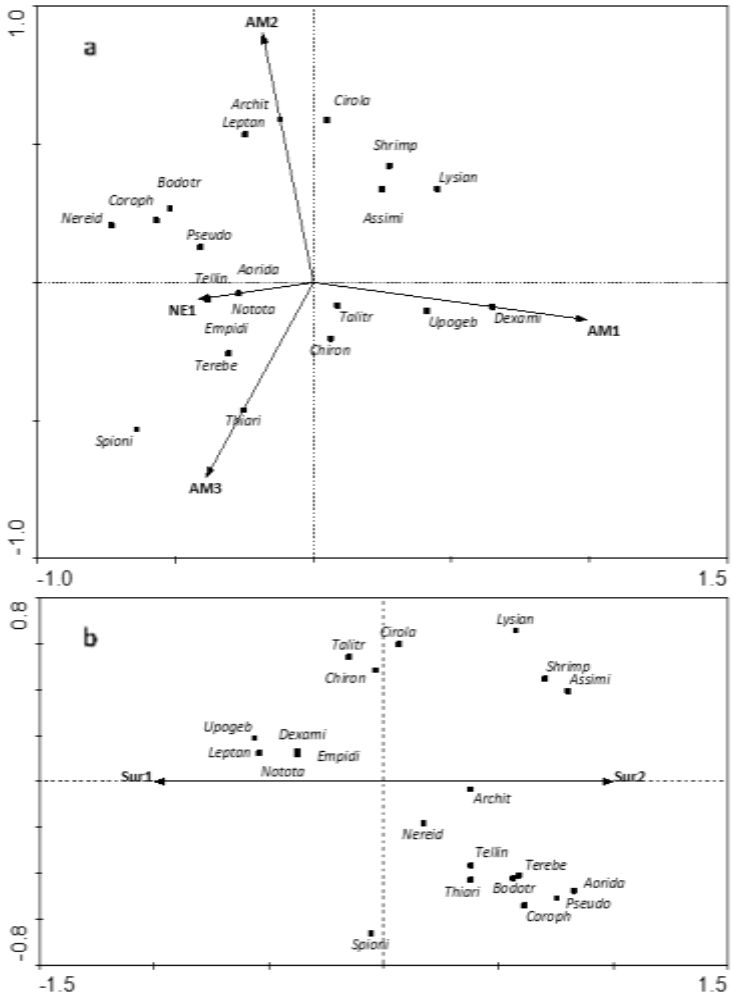

Figure 3.3 RDA ordination of estuarine macrobenthic community assemblages in relation to (a) the sampling sites (variance on the 1st axis was 52.5 % and an additional 32.6 % on the 2nd axis) and (b) the sampling surveys (variance on the1st axis was 100 %) in the Amatikulu/Nyoni Estuary.

The third run of statistical analysis to evaluate if there was a relationship between the Amatikulu/Nyoni Estuarine macrobenthic community assemblages and sediment grain size, there was no relationship (Figure 3.4a; $p = 0.812$). According to the ordination plot (Figure 3.4a), the

most important factors controlling the macrobenthic community are the very fine and fine grain sizes because the majority of the macrobenthic community clustered around them. The fourth run of statistical analysis using Canoco 4.5 was to evaluate if there was a relationship between the estuarine macrobenthic community assemblages with water quality variables and TOC (Figure 3.4b). There was a high correlation of water quality variables (TOC, SRP, Chlorophyll a, pH and TP). In the manual and automatic selection, ORP was the only water quality variable that had a significant p-value (0.04). Salinity levels at AM1 decreased during the 2nd survey whereas the salinity levels increased for the other three upper estuary sites (Table 3.2). There was more variation in the sediment grain size distribution during the second survey (Table 3.2).

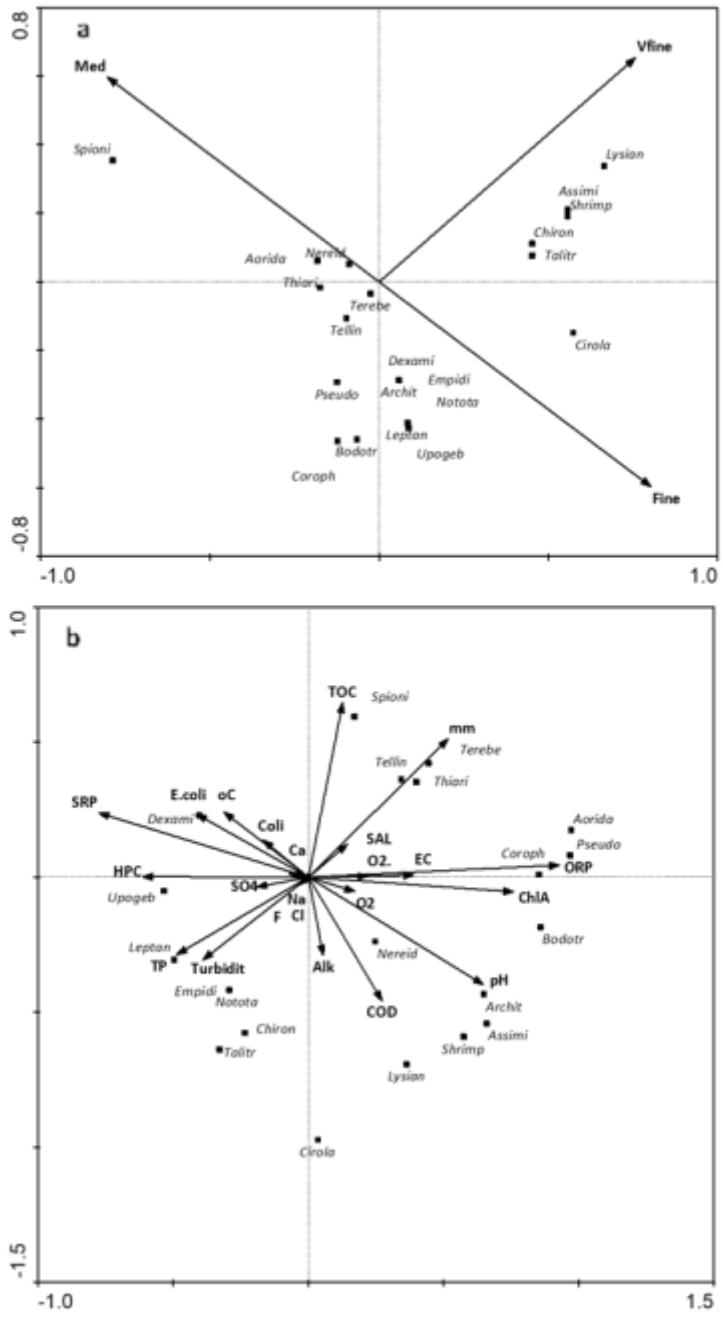

Figure 3.4 RDA ordination of estuarine macrobenthic community assemblages in relation to the (a) sediment grain size (variance on the 1st axis was 60.1 % and an additional 39.9 % on the 2nd axis) and (b) water quality and TOC (variance on the 1st axis was 43.4 % and an additional 19 % on the 2nd axis) in the Amatikulu/Nyoni Estuary.

Table 3.1 Macrobenthic invertebrate abundances of the Amatikulu/Nyoni Estuary sites collected over two sampling periods in 2017. The suffix 1 and 2 for the estuary site codes represent the 1st and 2nd survey respectively. (See methods for full site names)

Taxa	**AM1_1**	**AM2_1**	**AM3_1**	**NE1_1**	**AM1_2**	**AM2_2**	**AM3_2**	**NE1_2**
Dexaminidae	3							
Upogebiidae	2			1				
Nereididae	1	232	68	179	51	70	91	97
Leptanthuridae		165		10				
Cirolanidae		3		20	9	17		
Corophiidae		3		2		69	104	13
Terebellidae		2			1		31	13
Aoridae			5		3	202	115	53
Spionidae			138	4		1	3	25
Talitridae				3	1			
Chironomidae				29	6		1	
Bodotriidae				10		237	13	33
Empididae (larvae)				19				
Nototanaidae				1				
Pseudodiaptomidae						30	18	6
Shrimp larvae					6	10		1
Lysianassidae					8	5		
Assimineidae					42	75	1	3
Architectonicidae						2		
Thiaridae							1	
Tellinidae								2
Total number of individuals	6	405	211	278	127	718	378	246
Number of taxa	3	5	3	11	9	11	10	10
No. of individuals per 0.026 m^2	250	16875	8791,667	11583,33	5291,667	29916,67	15750	10250

Table 3.2 Water quality variables of the Amatikulu/Nyoni Estuary sites collected over two sampling periods in 2017. The suffix 1 and 2 for the estuary site codes represent the 1st and 2nd survey respectively.

	AM1_1	AM2_1	AM3_1	NE1_1	AM1_2	AM2_2	AM3_2	NE1_2
DO mg/L	10.2	10.07	15.46	10.61	8.62	15.47	9.48	8.65
DO %	129.9	113.4	168.9	122	99.1	179.8	110.8	102.2
EC	26.37	9.025	2.264	7.898	20.56	18.52	18.86	25.07
Temp	20.56	19.68	19.35	20.96	18.22	19.49	19.71	19.5
pH	6.96	6.38	5.65	6.59	7.63	7.9	7.63	7.31
Salinity	23.03	5.07	1.17	4.39	12.34	11.01	11.23	15.31
ORP	-17	-8.3	-14.9	-10.8	15.2	37.7	47.2	44.8
PHmV	-4.3	26.8	65.5	16.6	-64	-67.8	-63.8	-45.9
Depth (m)	1.2	0.7	0.6	1.1	1.25	1.35	1.6	2.5
Colour	Clear	Clear	Tea brown	Tea brown	Clear	Clear	Tea brown	Tea brown
Alkalinity	133	94.2	60.3	101	113	106	101	109
Cl	12236	2354	459	2237	6479	5232	4896	4691
NO2	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
NO3	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
SO4	1816	343	62.9	318	962	798	717	681
Ca	237	61.8	23.7	52	126	104	96	99
COD	940	708	132	214	2905	1425	1119	1008
Coliforms	>2420	980	>4839	>4839	1986	>2420	>2420	>2420
E.coli	1120	3	26	4	20	1	4	3
F	816	312	167	312	448	389	366	353
HPC 37	132	>1000	>1000	>1000	143	89	125	189
Na	5810	1306	333	1272	3225	2595	2405	2335
NH3	0.49	<0.10	<0.10	<0.10	0	0	0	0
SRP	12.9	13.3	17.8	14.2	<5.00	<5.00	<5.00	<5.00
TP	34.3	73.7	52.7	169	22	19	29	36
Turbidity	9.4	4.7	10.3	47.7	3	3	3	6
Chlorophyll a					<0.14	2	0	2
Coarse sediment	0.100	0.000	10.100	0.100	0.020	0.093	2.632	1.928
Fine sediment	99.900	100.000	89.900	99.900	99.980	99.907	97.368	97.684

Very fine sediment	0.000	0.000	0.000	0.000	0.880	0.445	0.294	0.388
TOC	2.340	3.910	4.060	2.560	1.790	1.550	4.610	4.300

3.4 Discussion

We had predicted that the main drivers of the macrobenthic invertebrate community assemblage structure and composition would be the sediment grain size distribution and salinity, but this was not the case in our study. For our study we identified 21 families in the Amatikulu/Nyoni Estuary for both the sampling seasons combined which correlated with the number of families Teske and Woolridge (2001) found in their study. In our study there was no relationship between the macrobenthic community and sites, however the mouth site (AM1) was the most dissimilar in comparison with the other three sites as it had the lowest diversity and abundance of taxa. In other studies, the mouth site generally had a different species composition (such as Family Dexaminidae) than the upper sites which may be due to the varying environmental surroundings (Carvalho *et al*., 2005; Whitfield *et al*., 2008). According to Teske and Woolridge (2001) temporary open/closed estuaries (TOCEs) in South Africa should have relatively fewer species and Carvalho *et al*. (2005) and Whitfield *et al*. (2008) had lower species abundance and diversity at mouth sites in comparison with other upper estuary sites. This was clearly shown in the ordination plot for our study. This may be a result of a stable or resilient community structure (Whitfield *et al*., 2008). NE1 and AM3 appeared to be the most similar sites possibly due to being relatively close in distance and having similar environmental conditions.

We found there was a significant relationship between the macrobenthic invertebrate communities and the sampling surveys. Survey 1 was conducted when the mouth was open after heavy floods due to Cyclone Dineo and when the mouth was closed during survey 2. It has been documented that when the mouth is closed in a TOCE, the habitat is relatively stable; water levels are higher; and more species are available due to more stable conditions (Whitfield *et al*., 2008). Survey 2 was conducted in spring which has been documented as a time when there is high juvenile recruitment in South African estuaries, this may also contribute to the increased number of individuals during the sampling session (Kalejta and Hockey, 1991). This may also explain why there were more species clustered around survey 2 in the ordination plot which was a good representation of the data with 100% variation shown on the 2D plot. The shrimp larvae were clustered around survey 2 when the mouth was closed which may be due to shrimp recruitment mostly occurring in these conditions (e.g. more habitat available) and when the estuary receives overwash (sea water coming in over the barrier) (Bernard and Froneman, 2005; Froneman, 2006; Whitfield *et al*., 2008). Generally, fewer species are expected after heavy rains or floods resulting in lower species diversity and abundance in estuaries (Owen and Forbes, 1997; Dittman *et al*., 2015) which has been well represented in our study with less species clustered around survey 1.

We found that there was no relationship between the macrobenthic community and sediment grain size, however, the majority of the Amatikulu/Nyoni Estuary macrobenthic community favoured very fine to fine sediment grain sizes. This may be due to little change of sediment grain size distribution over the two sampling sessions with little response from the macrobenthic community. Sediment grain size has been viewed as one of the most important drivers in macrobenthic community assemblages (Snelgrove and Butman, 1994; Dittman *et al*.,

2015). However, sediment grain sizes or any one environmental variable cannot solely be responsible for macrobenthic community assemblages (Snelgrove and Butman, 1994; Carvalho *et al*., 2005). Snelgrove and Butman (1994) noted that the top layers that are sampled are considered to be more homogenous due to bioturbation, therefore a sediment analysis is less likely to show a significant relationship with macrobenthic invertebrates. We sampled surface sediments in our study, the homogeneity of sediments mentioned in Snelgrove and Butman (1994) study may explain why there was no relationship between the macrobenthic invertebrate community and sediment grain sizes. Muniz and Venturini (2001) noted that bottom sediments are heterogeneous due to transportation of sediment by tidal action and freshwater flow, this would provide a variety of habitats for macrobenthic invertebrate communities. Macrobenthic families, such as Polychaeta which was the most dominant family in our study making up 42.5 % of individuals identified, they play a role in the bioturbation of sediments within estuaries which breaks down sediment aggregates and redistributes nutrients (Hutchings, 1998).

Water quality and TOC were assessed in relation to the Amatikulu/Nyoni estuarine macrobenthic community structure and distribution. The variables with a high correlation and had more influence on the macrobenthic community were mainly nutrients, which also accounted for the variation in the community structure. Dittman *et al*. (2015) noted that floods may change salinity levels and other water quality constituent concentrations, which affect macrobenthic community structures and species diversity. Nutrient concentrations have been known to peak after heavy rainfall events (Simpson and Hemens, 1978). With the estuary mouth closure or high freshwater inflow, there would also have been higher nutrient loading which, may be beneficial to the macrobenthic community if there were relatively low salinity and dissolved oxygen levels (Dittman *et al*., 2015) and in these conditions were represented in our

study. Water quality constituents namely total phosphorus (TP), soluble reactive phosphorus (SRP) and TOC had the most influence on the macrobenthic community structure and diversity. Increased nutrient levels may result in an increase in chlorophyll a which is associated with degraded water quality (Dauer *et al*., 2000), however TP, SRP and chlorophyll a concentrations were relatively low (Dallas and Day, 2004). Low nutrient levels indicated there was low algal production, therefore eutrophication, and other high nutrients related threats do not pose a threat to the organisms in the system. Herman *et al*. (1999) noted that the accumulation of organic matter in sediment may increase the diversity and abundance in the macrobenthic community. In our study, AM3 and NE1 had an increase in sediment TOC from survey 1 to 2, which correlated with the increase in species abundance and diversity, similar to the study conducted by Herman *et al*. (1999).

During survey 1 when the floods had occurred, there were lower salinity levels which results from high freshwater inflow (Carvalho *et al*., 2005). We found that salinity values were higher and relatively similar across all the sites during survey 2 when the mouth was closed, due to less freshwater and salt water outflow and inflow, however there was overwash (Whitfield *et al*., 2008) which was observed in the afternoon when the tide would wash over the mouth barrier increasing salinity concentrations.

In conclusion, our study investigated the macrobenthic invertebrate communities structure and distribution in the Amatikulu/Nyoni Estuary and determined how their structure and distribution related to the surrounding environmental factors. Our study showed how a flood event and the permanence of the mouth in a TOCE can have an impact on the macrobenthic invertebrate community assemblages as in other studies (Kalejta and Hockey, 1991; Owen and Forbes, 1997;

Dittman *et al*., 2005; Whitfield *et al*., 2008). Furthermore, our study was able to highlight that there was a change in species abundance and diversity along a gradient whereby the upper/inner reaches of the estuary supported more species than the lower reaches (mouth). This pattern has also been documented by other studies (Carvalho *et al*., 2005; Whitfield *et al*., 2008). Our prediction that the main driver of the macrobenthic community assemblages would be the sediment grain size distribution and salinity was not supported. The major drivers in this study were the nutrient constituents such as TOC. In our study there was no relationship between the macrobenthic community assemblages with sediment grain size and salinity although previous studies (Snelgrove and Butman, 1994; Dittman *et al*., 2015) viewed these environmental variables as major community drivers. Overall the Amatikulu/Nyoni Estuary supported a variety of macrobenthic communities. However, more research is needed over an extended period of time to determine how these macrobenthic communities change with time.

3.5 Acknowledgements

Special thanks to the National Research Foundation (NRF); Aquatic Ecosystems Research group (AER), Ford Wildlife Foundation, and the South African Pulp and Paper Industries (SAPPI) for financial support. Thank you to M. Vezi for data collection guidance.

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CHAPTER 4

CONCLUSIONS

We undertook a baseline study of the lowland Amatikulu River catchment because relatively little is known about its current ecological status. Unknown catchments are being developed and their resources used without any monitoring. What is needed for these systems is knowledge on what can be managed and how it can be managed and if we should. Baseline studies for the evaluation of the ecological condition of an ecosystem using aquatic macroinvertebrates as indicators of ecosystem health, are needed to inform management decisions. Knowledge from this thesis will contribute to the understanding of the freshwater and estuarine macroinvertebrate communities of the lowland Amatikulu catchment.

The SASS5 tool was useful for the evaluation of the levels of pollution to the freshwater sites, especially since it was developed specifically for South Africa (Chutter, 1994; Dallas, 1997; Dickens and Graham, 2002) and used in neighbouring countries because it has been working well (Gratwicke, 1998). The ecological condition of the upper freshwater sites (Chapter 2) showed that the waterways in this region were in a relatively good state (B/C). This may be due to low impact anthropogenic land use activities such as small scale subsistence farming and rural dwellings, whereas the lower freshwater sites were surrounded by intensive sugarcane farming which likely increased organic nutrient levels that could result in eutrophication and more settlements which contribute to the degradation of the river health (Nhiwatiwa *et al*., 2017). The more intensive anthropogenic land use activities have resulted in the lower freshwater sites being in a modified or degraded state (C/D). The overall ecostatus of the freshwater system of the lowland Amatikulu catchment was C/D during our study period.

We identified some environmental variables such as TP, TOC and mouth permanence as playing a role in controlling the macrobenthic community structures in the Amatikulu/Nyoni Estuary (Chapter 3). Mouth permanence has been documented to be affected by river inflow (Dix *et al*., 2008; Hanekom and Russell, 2015; Ortega-Cisneros *et al*., 2016). It reflected in our baseline study as there was a difference in the macrobenthic community structure when the estuary mouth was either open or closed. In our study nutrients may have played a large role in controlling the macrobenthic community structure due to surface runoff from intensive sugarcane farming around the estuary and further upstream (Dauer *et al*, 2000). Although sediment grain size distribution did not have a significant relationship to the macrobenthic communities present, we were able to highlight that the Amatikulu/Nyoni Estuary macrobenthic communities appeared to prefer fine to very fine sediment grain sizes.

To conclude, sampling within the lowland Amatikulu catchment for the study was very informative about the macrobenthic communities found there. However, sampling annually would further improve the knowledge and depict annual variability in the lowland catchment's ecosystem. Monitoring over a longer period of time can inform best practices suited for the lowland Amatikulu catchment. It is recommended that the freshwater system of the lowland Amatikulu/Nyoni catchment continue be monitored by using the SASS5 tool (Dickens and Graham, 2002). In addition, the estuarine system of the lowland catchment should continue to be monitored by using lines of evidence such as species abundance and the Shannon Wiener diversity index, because there is no specific tool in South Africa used to monitor estuarine health using macrobenthic invertebrates as indicators of ecosystem health. Currently the legal requirement to maintain a sustainable balance between the use and protection of water resources in KwaZulu-Natal (National Water Act, 1998) and protect biodiversity (National Environmental

Management Act of 1998) appears to be leaning towards use and not to the protection of water resources within the lowland Amatikulu catchment (pers. obs.) so this needs to be addressed. An Estuary Management Plan (EMP) for the Amatikulu/Nyoni estuary should be established and implemented. More detailed analyses should be undertaken in the lowland Amatikulu catchment where anthropogenic threats to the wellbeing of ecosystems are relatively high. Many unauthorised land use practices were identified during the study including habitat alterations and water abstraction for example, and these should be addressed. Collaboration between stakeholders of the protection of water resources in the lowland Amatikulu catchment should be promoted, capacity building and awareness is urgently required amongst stakeholders to bridge the gap between protection and use of this important system.

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